

The University of Chicago
FOUNDED BY JOHN D. ROCKEFELLER

THE MORPHOLOGY OF ELODEA CANADENSIS

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL
OF SCIENCE, IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF BOTANY)

BY
ROBERT BRADFORD WYLIE

CHICAGO
1904

581.24
W 97
paw

PRINTED AT THE UNIVERSITY OF CHICAGO PRESS

BOTANICAL GAZETTE

JANUARY, 1904

THE MORPHOLOGY OF *ELODEA CANADENSIS*.
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY.
LII.

ROBERT B. WYLIE.

(WITH PLATES I-IV)

THE Helobiales occupy a place of special interest among monocotyledons. Beginning with members having the simplest flowers, the group includes an ascending series of forms and finds its climax in the Hydrocharitaceae which display considerable floral complexity. This series has attracted much attention from plant morphologists and early investigation naturally centered among the simpler members. It was with the hope of adding something to the data concerning one of the higher forms that this study was undertaken. *Elodea* further invites attention on account of its being one of the most specialized of submersed aquatics.

My thanks are due Professor John M. Coulter and Dr. Charles J. Chamberlain for kindly suggestions and valued assistance.

FLORAL DEVELOPMENT.

The flowers of *Elodea canadensis* are usually borne singly in the axils of leaves, and are scattered along the stem in a loose indeterminate inflorescence. They are so far apart, however, being separated by fifteen to twenty internodes, that one flower is well developed before the primordia of the next younger one are established.

The flowers are functionally monosporangiate, though rudiments of the suppressed parts are often present. Three sterile

stamens are commonly found in the pistillate flower, and six have been reported to occur in rarer instances. These staminodia are composed of uniform parenchyma and show no evidence of the suppressed sporangia. Much more unusual is the development of stigmas upon the staminate flower. Eichler (5) refers to these as being possibly rudimentary stamens; the writer, however, has found well-developed stigmas above the nine stamens usually borne by the staminate flower. In one instance the stigma was clothed with papillae and covered with pollen grains which had sent their tubes into its tissue. In such a case it would appear that the usual stigmatic secretions are present and that it differs in no essential respect from a normal stigma. Such conditions recall Chamberlain's (2) discussion of the teratology of *Salix*, in which monosporangiate, dioecious genus he reports almost all possible combinations in the production of the sporangia.

The occurrence of these rudiments in *Elodea* must point back to an ancestry with perfect flowers, and their specialization has no doubt been correlated with the changed conditions of the submersed habitat. The flowers are now dioecious and are peculiarly adapted to the combined influences of wind and water for pollination. The pistillate flower is quite complex and presents one of the most striking cases of epigyny known; the staminate flower is simpler and has acquired the habit of breaking loose from the stem at maturity.

The development of the pistillate flower was studied by Horn (3), who also investigated the vegetative plant body; but since his work was based on the external aspects or rough sections only, the account, while accurate in a very general way, lacks most of the details secured through modern methods. The pistillate flower begins as a protuberance from the side of the stem near the growing point (*fig. 1*). This swelling pushes out rapidly, soon equaling the stem tip in length and giving it the appearance of having bifurcated (*fig. 2*), but the main axis soon reasserts its dominance, leaving the flower as a distinctly lateral member (*fig. 3*). At this time ridges near the base of the flower mark the origin of the spathe (*fig. 2*), which pushes out rapidly and for a long time envelops the developing flower (*figs. 2-12*).

The apex of the receptacle now flattens and broadens slightly, and, with the earliest indication of the floral parts, a cylindrical mass of tissue grows up (*figs. 5-10*), leaving a triradiate slit down its center, the walls being closely pressed together. The various parts of the flower continue meanwhile to develop at the outer end of this rapidly elongating growth. The calyx pushes out first (*fig. 5*), the sepals soon curving over the growing parts within; next comes the whorl of three sterile stamens (*figs. 8-9*), followed by the three stigmas (*figs. 10-12*); last of all appears the corolla (*figs. 11-12*).

Simultaneous with the development of the stigmas at the outer end of this floral tube is the growth of the ovules within its base. These push out from the surfaces of the central opening (*fig. 10*), but it is only as they develop that traces of the ovarian cavity appear (*fig. 11*). The walls which have previously remained in contact are now pushed apart, forming the rounded triangular ovary (*fig. 12*). The parts above ultimately coalesce, roofing over the cavity.

The various parts of the flower having been established, the floral tube, that region of fused parts between ovary and sepals, enters upon a period of more rapid elongation. The direction of growth is at first a negative geotropic response, and the young flowers stand up quite stiffly, but during later development proper orientation is probably due entirely to the buoyancy of the enclosed gases. Very early in the history of the flower the beginnings of three rows of air spaces can be seen, extending through its whole length (*fig. 11*). These start as rifts between cells and increase in size with the growth of the flower (*fig. 12*), until at maturity they constitute a large part of its volume. It is the low specific gravity of the floral tube that insures its direction toward the surface of the water, as these parts are very weak and cannot support themselves. A cross-section of the floral tube shows an outer ring, composed of two layers of cells, and a central conducting strand joined to the outer part by three bridges of tissue, leaving the three rows of air spaces between. The outer wall has no stored food, but all other cells are richly provided with starch.

The adult pistillate flower presents unusual proportions; the diameter of the floral tube is about 0.3^{mm} , while its length in extreme cases may be over 30^{cm} , in such an instance the flower being one thousand times longer than wide. The lower part is invested by the spathe, which is 2 to 3^{cm} long. The sessile ovary at the time of pollination is about 4^{mm} long and less than 1^{mm} in diameter.

The staminate flower originates in the same general manner as the pistillate, and in early stages they might easily be confused. The later development of the pollen-bearing flower differs markedly, as it shows none of the complexities described above. The receptacle, instead of pushing up into a floral tube, becomes merely conical, and gives rise in turn to sepals, outer stamens, inner stamens, and very much later the corolla, which is not prominent and may be quite rudimentary (*figs. 13-22*). A conical protuberance is often seen in the center of the flower to which the inner stamens may be adnate for a part of their length (*fig. 22*). That part of the pedicel between the insertions of spathe and sepals elongates slightly and develops large air chambers (*fig. 22*). It is at the upper end of this region that the break occurs which sets free the flower at maturity. The exact mode of detachment was not determined.

THE FEMALE GAMETOPHYTE.

The female gametophyte is developed while the floral tube is elongating toward the surface of the water. The tube had a length of about 4^{mm} when the primary sporogenous cell was established; was twice that long when the ovule contained a 2-celled embryo sac; and had attained a length of 15^{mm} at the 8-celled stage.

The single archesporial cell (*fig. 23*) cuts off a primary parietal cell which ordinarily divides first by an anticlinal wall (*fig. 24*). The details of development of the parietal tissue were not studied, but it seems to be very limited in amount and probably does not persist long.

The primary sporogenous cell enlarges greatly, and in its division the spindle lies wholly in the outer half of the cell (*fig. 25*). The resultant cells differ greatly in size, the deeper one

being six or eight times larger than the micropylar one. In the division of these daughter-cells the spindles show a corresponding difference in size and vary in direction, especially in the outer cell, in which it may lie at right angles to the longer axis of the ovule (*figs. 26, 27*). Four megaspores are usually formed, which are separated by walls. In one instance it was observed that the two central cells of the row of four had each divided again, resulting in six megaspores (*fig. 28*). This case was made very clear as the megaspore at either end of the row had enlarged, thus lessening the possibility of confusing sterile cells with megaspores. This particular row is of interest also in showing a struggle for dominance between the innermost and outermost cells. In all other cases noted there were but four megaspores, and the innermost one seems regularly to be the successful one (*fig. 29*).

With the division of the megaspore there is introduced a change in the form of the embryo sac, which, though often seen in later development, is seldom introduced at so early a stage. The upper one-third of the embryo sac begins to enlarge, and by the time this first division is completed has a diameter nearly twice that of the cylindrical part beneath (*fig. 30*). As development proceeds this differentiation becomes more marked. The outer part, in which all the growth takes place, continues to enlarge, being at first spherical, then oblong, and finally assuming the usual embryo sac form. The inner part persists with dimensions unchanged or somewhat diminished by encroachments of the main body of the embryo sac (*figs. 31-36*). While such a pouch-like antipodal end is not uncommon, the emphasis laid on its early development in this instance might suggest its being a rudiment of a once prominent nutritive device, but it probably functions now in no important way.

At the two-celled stage one nucleus passes to each end of the sac (*fig. 30*). The divisions giving rise to the antipodal group occur deep within the pouch (*figs. 31, 32*). The spindles seen here are much smaller than those of the micropylar end, and the resultant nuclei of the two groups show a proportional difference in size (*fig. 32*).

The antipodal polar appears regularly to issue from the pouch at an early stage, and as it passes into the body of the embryo sac it increases in size, until at the time it arrives in the vicinity of the other polar no difference in their dimensions is discernible (*figs. 34, 41, 42*). The polars soon approach one another and may remain for a long time in contact. Their actual fusion was not observed and seems not to occur until the time of fertilization. Shibata (14) has shown for *Monotropa* that pollination hastens the fusion of the polars. In *Elodea* neither pollination nor the entrance of pollen tubes into the ovary constitutes a sufficient stimulus. In ovules which have failed to receive pollen tubes the polars may still be seen lying side by side, even though surrounded in the same ovarian cavity by other ovules whose eggs have been fertilized, and which contain embryos. The actual presence of the pollen tube in the ovule seems to be necessary to bring about their union. Guignard (9) has shown a similar behavior of polars in *Capsella*. In this form the polar nuclei are distinct until the male elements penetrate into the embryo sac; their union appears to be accomplished, however, before fertilization takes place.

The antipodals always remain in the pouch in which they were formed, and before the entrance of the pollen tube are inconspicuous and stain feebly. At the time of fertilization, however, and during the early development of the embryo, some activity is manifest in this region. The group then generally shows a fourth nucleus (*figs. 35, 36, 38, 39*), the uppermost of which becomes quite large and may have more than one nucleolus. This enlarged nucleus is often surrounded by a considerable mass of cytoplasm which may be enclosed by a definite membrane, giving it the appearance of an egg (*figs. 38, 40*). In rarer instances two enlarged nuclei are seen, making five in all (*fig. 37*), while not uncommonly the tip displays the usual number, three (*fig. 40*).

The sudden appearance of the extra nucleus in the antipodal group, when one has in mind the behavior of the polars, might suggest that these nuclei do not always fuse, and that one of them passes down to the lower end of the embryo sac and joins

those in the antipodal pouch. The general evidence, however, is against this view. The irregular number of nuclei displayed here and the general arrangement of the cytoplasm about them would indicate that any increase in number has come from divisions among the antipodals—an activity in this region that often results from fertilization. In all embryo sacs studied at earlier stages the lower polar had passed out of the tip, and its return to the antipodal group seems here improbable.

THE MICROSPORANGIUM.

The stamens are very short-stalked, the sporangia being practically sessile, and are further characterized by a relatively small amount of sterile tissue. Each stamen develops only two sporangia, there being no suggestion of a greater number at any stage. This condition seems to occur regularly in but a limited number of forms. The presence of only two sporangia to the stamen has long been known for the Asclepiadaceae, and has recently been reported by Shoemaker (15) for Hamamelis. The occurrence of this phenomenon in such widely separated and differently specialized forms offers no clue to its significance.

The developing stamen is at first circular, later becoming oblong in cross-section. From a homogeneous meristematic mass there differentiates at either side of the stamen a hypodermal archesporial column (*fig. 43*). These cylinders of tissue extend the whole length of the stamen and have from five to eight cells each in cross-section. They are separated by a mass of sterile cells which later develops the rudimentary bundle and contributes somewhat to the tapetum of both sporangia. The archesporial cells become quite distinct, differing from these sterile cells in size and staining reactions.

In the establishment of the primary wall layer the divisions of the archesporial cells are not simultaneous, the outer ones tending to divide a little earlier than those nearer the body of the stamen. It follows, from the form of the stamen and the extent and location of the archesporium, that the primary wall layer nearly invests the column of primary sporogenous cells, and this investment is made more nearly complete in places by the

introduction of curved walls at the axial corners of the archesporium (*fig. 44*). While in no observed instance was the inclosure quite complete at this time, it was noted that a little later the cells on the axial side may divide (*fig. 45*), thus bridging the gap in the wall layer. Coulter (3) has shown that in *Ranunculus* a part of the tapetum may come from the sporogenous tissue. Rosenberg (12) finds in *Zostera* that the tapetum on both the inner and outer sides can be traced back to the divisions of the greatly elongated sporogenous cells. In *Elodea* these divisions of sporogenous cells on the axial side seem to be of common occurrence, and there is probably a regular contribution to the tapetum from the sporogenous cells in that region. In addition there may be a contribution to the diffuse tapetum on any side by the sacrificing of potential spore mother-cells to the nutritive function. When the time of spore formation is at hand the functioning mother-cells are fewer than were the primary sporogenous cells, notwithstanding divisions may often take place among these tending to increase their number. The successful spore mother-cells become greatly enlarged before their division (*fig. 47*). A cross-section of the sporangium at this time may show only one of these cells, the others having broken down, though commonly there are three side by side (*fig. 46*), and portions of four are often seen in a given section.

The primary wall cells form a zone several layers of cells in thickness, of which the outer one only, the endothecium, persists until the discharge of the spores.

THE MALE GAMETOPHYTE.

The male gametophyte is considered to have begun with the spore mother-cells. Both the first and second divisions of the mother-cells are characterized by slender curved spindles terminating at either end in the plasmatic membrane (*figs. 48, 49*). Such spindles have been figured by Strasburger (16) for *Ceratophyllum*. Following the first division a delicate wall is formed, dividing the mass into two hemispherical cells. The nuclei of these daughter-cells are greatly elongated. The number of chromosomes was not made out with certainty, but is probably twelve for the gametophyte.

In the second division the spindles commonly lie parallel, in which case the four spores of the tetrad develop in one plane; but the spindles may be rotated so that their planes intersect at right angles, resulting in a correspondingly different grouping of the pollen grains. Following the second division, the four microspores are organized in the usual manner. The young spores lie for a time within the wall of the mother-cell (*fig. 50*), but the small tetrads soon appear naked (*fig. 51*) and enter upon a period of rapid enlargement. The four members of the tetrad do not fall apart, but remain attached and are ultimately shed from the sporangium still firmly joined together. This union is so intimate at maturity that violent shaking in a closed vessel partly filled with water seldom breaks them apart.

Though borne by one of the most specialized of submersed aquatics, a plant entirely devoid of cutinized walls in all its vegetative parts, the microspores of *Elodea* exhibit a strongly cutinized exine and a well-developed intine. In this connection it is interesting to note that in *Najas* and *Zannichellia*, also submersed aquatics, Campbell (1) finds no exine developed. Strasburger (16) reports for *Ceratophyllum*, which has a similar habitat, a pollen grain without intine and with a thin smooth exine which is cutinized, but not nearly so strongly as in air blooming plants. In *Elodea* the exine closely resembles such coats borne in normal aerial sporangia, and is beset with multitudes of spines, which play an important part in the process of pollination, as we shall see later. These spines (*fig. 67*) are cylindrical, with conical points barbed at the base and each bearing at its tip a tiny disk.

The intine possesses in numerous instances peculiar thickenings reaching into the cavity of the spore. These ingrowths may be merely papillae, though they are often rod-like, or even membranous, forming trabeculae extending well across the pollen grain. These protrusions are integrated with the intine at their junctions, and appear to be perfectly uniform in character and substance with it.

The microspore nucleus divides long before the pollen grain has attained its full size (*fig. 52*), and at a time when the spore wall is not yet differentiated into two layers. This first division

appears to be simultaneous in the four spores of a given tetrad (*fig. 53*), though widely varying stages may be found within a single sporangium. The spindle fibers in this division after the cell plate is laid down are much more prominent on the tube nucleus side (*fig. 54*). The generative cell when first cut off along the wall of the spore is crescentic in outline, with a greatly elongated nucleus, but it soon becomes lenticular in form. Later, after its passage into the cytoplasm of the tube cell, it is for a time spherical (*fig. 55*); subsequently it becomes greatly elongated, and just before its division into the male cells is curved and may extend nearly across the spore (*fig. 57*).

The tube nucleus enlarges soon after its formation so as to become very conspicuous. It is at first spherical (*fig. 54*), but a little later becomes amoeboid in shape and assumes the most varied forms, accompanied by a changed reaction to stains (*fig. 56*). Shortly before the division of the generative cell the tube nucleus regains its original form; with the organization of the male cells, however, it may again exhibit irregularities of outline. Schaffner (**13**) in his studies of *Erythronium* found the generative cell very large and displaying such general activities as are here associated with the tube nucleus.

The formation of the male cells seems to occur regularly long before the pollen grains are shed from the sporangium. The spindle formed within the long crescentic generative cell is itself slightly curved (*fig. 58*), and the daughter nuclei retreat quite far apart before the partition is formed between them (*figs. 61, 62*). The male cells when first organized remain for some time end to end in the relation occupied at the time of their formation, and thus continue the bow-shaped outline which characterized the generative cell. Subsequently, with the symmetrical elongation of both male cells, their adjoined ends become long drawn out, yet still remain attached by their tips. This point of union acts like a hinge, permitting the cells to take the most varied positions with respect to one another, even swinging about so as to lie side by side (*figs. 63-66*). In no observed instance did the male nuclei lie far enough apart to preclude the possibility of their cells being still united by these elongated

ends. As will be seen later, there is reason for believing the male cells of *Elodea* make the greater part of the journey through the pollen tube still hitched together in this tandem fashion.

During their continuance in the pollen grain the male structures clearly reveal their morphology as cells. About the nucleus, which usually shows a nucleolus, there is an extensive mass of cytoplasm differing considerably from the contents of the spore, and all clearly invested by a limiting membrane. It was observed that one or two more deeply staining bodies usually lie outside the nucleus along the median line of the cell (*figs. 64, 65*); if two of these are present, one lies at each side of the nucleus, though at varying distances from it.

PHENOMENA OF POLLINATION.

While the general mode of pollination in *Elodea* is well known, the details, which seem never to have been published, are of such interest as to merit a brief description.

The staminate flowers are borne entirely beneath the surface of the water, and these, as is well known, break off and rise to the surface, there shedding the pollen. It is probable that with the ripening of the sporangia, in the still submerged flower, gases given off by the plant fill the spaces about the spores as well as any other cavities developed in the flower. At maturity a bubble of oxygen forms at the tip of the flower, and with its enlargement the sepals open slightly. At this time, looking down into the flower one can see that the sporangia have opened, and that many of the spores have been shed into the central space. The oxygen bubble may finally become nearly as large as the flower, and, when conditions are proper, the buoyancy of the enclosed gas, aided by the low specific gravity of the flower itself, overcomes the weakened attachment, and the flower darts to the surface. Upon reaching the surface the bubble disappears, the sepals snap back quickly, and in their recurved position form three boat-like floats which support the sporangia above the water; these catch the breeze and the flower sails away. While such float devices for the staminate flower are thought to be of great importance in the pollination of *Vallisneria*, it is doubtful

if any significance can be attached to them in *Elodea*. The pollen was nearly all discharged at the moment the flower came to the surface, and any remaining portion would have no better opportunity for reaching the stigma of the pistillate flower. The snow-white tetrads are quite conspicuous floating on the water, or scudding along the surface with the wind.

The floating of the pollen grains is due to the nature of the outer spore coat. In a previous paragraph it was mentioned that the exine was covered with spines, each bearing at its tip a slight enlargement; these spines tend to hold back the surface film from contact with the body of the spore, and thus imprison enough air to keep it afloat. The microspore has a greater specific gravity than water, and will sink at once if wetted. This can be demonstrated easily by placing the spores in dilute alcohol, then transferring quickly to water. A simpler and more striking method is to float a quantity of these spores on the surface of water half filling a stender dish; then cover and shake vigorously for a moment. The violent agitation of the water breaks the surface film, permitting the liquid to come into more intimate contact with the body of the spores, which sink at once to the bottom of the vessel.

While the gas bubbles may not be necessary for pollination, they are certainly very helpful. Their buoyancy aids in detaching the flowers, raises them quickly to the surface, and the sudden recurving of the sepals may be related in some way to the escape of the bubbles on reaching the air. The accumulation of gas about the spores in the submerged flower is also of significance in that it prevents the moistening of the ripe spores while yet submerged; for this, as we have seen, would lead to their sinking upon release. The general relation of these accumulations of oxygen is shown by the fact that in quiet waters of aquaria the rapid appearance of the staminate flowers at the surface of the water is simultaneous with the first marked photosynthesis of the day. During the early part of the forenoon the flowers which have ripened during the hours of darkness are rapidly brought up as the liberation of gas by the plant is increased, and very few appear in afternoon or evening.

The pistillate flower, as has been noted above, reaches the surface of the water by the lengthening of the fused parts above the ovary. Elongation does not cease immediately upon reaching the surface, as there is usually developed some surplus length before the floral parts open. These parts are repellent to water and so resist wetting for many hours. With the opening of the flower the three prominent stigmas quickly recurve, arching well out over the floral envelopes. Lying thus, commonly on its side at first, the weight of the flowers rests chiefly upon the stigmas. Since the stigmas are not readily wetted by water, they form a depression in the surface film. Pollen grains floating near the flower therefore approach and quickly slide down into contact with the stigma. There is thus established about each flower "a circle of influence," which in quiet waters is about 2^{cm} in diameter, and spores floating into this area are immediately brought into contact with the stigma. When numerous, pollen grains may form a layer lining the bottom of the depression. If at such a time the flower is submerged, as may happen from wave action, etc., the surface film binds together floral parts and spores as they pass beneath the water, and invests them with a considerable volume of air. When all again come to the surface the pollen grains may be seen sprinkled over all parts of the flower including the other stigmas. Spores lodged against any part of the flower may in this way be transferred to the stigmas.

It will be seen that the whole process of pollination is dependent in one way or another upon the *surface film* of water: (1) such a film makes possible the accumulation of oxygen bubbles within and above the staminate flowers, with whatever of advantage that may follow; (2) it is directly responsible for the floating of the pollen grains on the surface of the water; (3) the surface film brings the floating pollen grains into contact with the stigmas of the pistillate flower.

THE POLLEN TUBE.

As might be expected, considering the remarkable morphology of the pistillate flower, the study of the pollen tube was not without interest. These growths not only have great length, 5-30^{cm}, but are quite large and display some unusual activities.

While the formation of the pollen tube was not studied in detail, it seems to take place in the usual way. Microspores germinating on the stigma show the male cells still distinct (*fig. 69*). In no observed instance did all the spores of a group develop tubes; usually only one or two members of the tetrad germinate, the others being held back from the stigmatic secretions. The tubes show very prominently in cross-sections of the flower. They pass down the central conducting strand; occasionally one may be seen in one of the air chambers, but serial sections generally show it to be only a loop put out from the central tissue.

Having reached the upper end of the ovary, the pollen tubes pass with singular directness down through the ovarian cavity to the upturned micropyles of the ovules. One can but remark the efficiency of the chemotropism and the precision with which the tubes pass to the openings in the ovules (*fig. 75*). Few to many curved, tangled, and feebly staining pollen tubes are usually present among the fertilized ovules in the cavity. These seem to have come into the ovary too late; at any rate, the functioning pollen tubes are always much straighter and stain more deeply than the ones which have failed to enter the ovules.

It not infrequently happens that one of these functionless pollen tubes, having failed to enter a micropyle, may swell up at its tip and terminate its development in a cyst-like enlargement (*figs. 70-74*). Scores of these growths were encountered in material of all stages of development after fertilization, collected through two seasons from various stations, and killed in different ways. Their occurrence, therefore, is so uniform as to merit some attention, especially as they do not seem to have been discussed in connection with any other plant. These cystoids may be found lying anywhere in the cavity of the ovary, but seem never to occur in the style nor in any tissue through which the tube passes. They may lie along the walls of the cavity or in contact with the ovules, but usually are free in cavity of the ovary. In form they vary from spherical to oblong; some may be irregular in outline or even lobed, while those in contact with the wall or ovule are often much flattened or elongated (*fig. 74*).

These enlargements vary in size from slight swellings to growths ten or fifteen times the normal diameter of the tube. Their contents were studied with interest, as it seemed they might throw light on the nuclear conditions in the pollen tube. Their staining properties are uniform with those of the ordinary pollen tube, and nuclei are nearly always present, sometimes evidently disorganizing (*fig. 73*), but in other cases presenting a normal appearance. The tube nucleus was often quite conspicuous, but it was of special interest to note that in certain of these cystoids the male cells could be distinctly made out (*figs. 70, 71, 72, 74*). Each presented, under favorable conditions, its characteristic appearance. The ample cytoplasm was bounded by a definite membrane and showed within a distinct nucleus with its nucleolus. It was also noted that these male cells generally lie near one another, as though still joined together, and in certain cases this connection was very evident (*figs. 70, 74*).

The presence of the male structures as distinct cells in these cystoids lying among the ovules affords very definite information as to their condition during the journey through the pollen tube, and it is obvious that they have retained the features that characterized them in the pollen grain. Guignard (6) has contended that the male structures are found as cells in the pollen tubes of *Lilium*, though this is denied by Koernicke (4). More recently Guignard (9) has reported a similar condition for *Lepidium*, which unfortunately is accompanied by no satisfactory figure. Since the tubes producing these cystoids in *Elodea* may be longer than the functioning ones, and apparently differ from them in no essential respect, it is probable that, in this form at least, the male structures regularly maintain their integrity as cells until they come into the vicinity of the micropyle.

The pollen tubes of *Elodea*, furthermore, are remarkable for their persistence. Long after fertilization the enlarged tip of the pollen tube may be seen clearly outlined by the side of the suspensor cell (*figs. 78-83*), while stretching upward from the micropyle into the cavity of the ovary extends the tube still darkly staining and apparently turgid. These conditions persist until the embryo is well developed. Those portions of the tube

in the ovary more removed from the ovules show diminished contents or may be empty, but their course can be readily followed in the floral tube where the protoplasm is massed into the deeply staining "Propfen." The cystoids likewise remain long among the enlarging ovules, but their adjoined tubes, which are never conspicuous, soon collapse. All other functionless tubes seem quickly to perish in the ovary.

The male gametophyte as represented by the pollen tube, therefore, both in size and in length of life becomes a conspicuous generation. It is at first nourished by the stored foods of the spore and conducting tissue. After fertilization, with the growth of the ovary, connection is severed between the part¹ in the floral tube and that near the micropyle, the lower end continuing to live apparently parasitically on the embryo sac for a time.

FERTILIZATION.

The pollen tube is usually much contorted in its passage through the micropyle, and upon reaching the embryo sac it swells out in bulbous fashion, at the same time causing one of the synergids to disappear. The whole region is so disturbed by the vigor of the tube that the fate of this cell could not be certainly determined, but appearances indicate that the pollen tube may have passed into the synergid inflating it (*figs. 76-82*). It often happens that two pollen tubes pass into one ovule; in such cases both synergids disappear, and a favorable view of the embryo sac shows the two swollen, darkly staining tips of the pollen tubes arranged symmetrically side by side (*fig. 75*), as though growth of each had been stopped at a certain point. In one instance where three tubes had passed in through one micropyle the whole upper half of the embryo sac was filled by their contents. Guignard (8) has reported that in *Nicotiana Tabacum* and *Datura laevis* the pollen tube passes into one of the synergids.

The male cells were not seen in the embryo sac before fertilization. Numerous preparations showed one male nucleus in contact with the egg, and the fate of the second male nucleus was determined in a few cases when it was found uniting with

the endosperm nucleus (*figs. 35, 36*). In all instances but one the male nuclei seen in the embryo sac were in resting condition.

THE EMBRYO.

The oospore divides by a transverse wall. The upper nucleus retreats toward the micropyle and becomes the center of the vesicular cell, which later becomes enormously enlarged. The endosperm nucleus does not divide until a two-celled embryo is established. In many cases the oospore was found dividing with the endosperm nucleus still in resting condition (*fig. 76*); in other instances the endosperm nucleus was in mitosis, but the egg fully divided (*fig. 77*). Figures were never seen in both at the same time. Such a tardy division of the endosperm nucleus seems very rare. Guignard (7) has figured for *Naias major* the primary endosperm nucleus in the spirem stage by the side of a two-celled embryo. Hall (10) also finds in *Limncharis emarginata* two nuclei in the young embryo before "the upper polar, which forms the endosperm, has gone through the first division."

A proembryo of four or five cells is established before the end cell divides by a vertical wall (*fig. 79*). While the development of the embryo was not studied in detail, certain variations were noted in these earlier divisions. The basal cell becomes very prominent. When the parts of the embryo are established, this cell has a volume fully forty times that shown in *fig. 82*, and when the seed is nearly ripe it still shows prominently, though somewhat flattened, at the root end of the embryo (*fig. 83*). The remaining synergid also often enlarges for a time nearly as rapidly as the suspensor cell (*fig. 81*), and seems during the early stages to share with it a common function.

The embryo seems to be of the usual monocotyledonous type. The primary root probably does not function, and secondary roots are seen in the seed pushing out near the base of the stem (*fig. 83*).

SUMMARY.

1. The pistillate flower is strongly epigynous, and develops a long floral tube reaching from the sessile ovary to the surface of the water.

2. Four megaspores are usually formed; six were noted in one instance.

3. The embryo sac early develops a pouch in which the antipodal group of nuclei is formed.

4. The polars approach one another at an early stage and may remain for a long time side by side; their fusion, however, was not noted before fertilization.

5. The stamens regularly produce two sporangia each. The primary wall layer nearly invests the sporogenous tissue, which later may contribute to the tapetum on the axial side.

6. The pollen grains adhere in tetrads and have a greater specific gravity than water. The exine possesses spines which hold back the surface film and imprison sufficient air to keep the spores afloat.

7. The male cells are organized in the pollen grain and are joined together by their elongated ends.

8. Gas bubbles aid in detaching the staminate flowers and in bringing them promptly to the surface of the water.

9. The pistillate flower is impervious to water and so produces a depression in the surface film. Pollen grains floating near are brought into contact with the stigmas by means of gravity operating through the declined surface film.

10. The large pollen tubes, having penetrated the long floral tube, pass directly through the ovarian cavity to the upturned micropyles of the ovules.

11. Pollen tubes which have failed to enter ovules often swell up into cyst-like enlargements in the ovary. In these cystoids the male structures can be seen as distinct cells instead of nuclei only.

12. Fertilization takes place in the usual manner, and the second male cell was found uniting with the endosperm nucleus.

13. The primary endosperm nucleus does not divide until a two-celled embryo is established.

14. The pollen tubes persist until the embryos are well developed.

15. The suspensor cell of the embryo becomes enormously enlarged and the synergid often increases in size. The primary

root probably does not function, and secondary roots are developed in the seed from the lower parts of the stem.

THE UNIVERSITY OF CHICAGO.

LITERATURE CITED.

1. CAMPBELL, D. H., A morphological study of *Naias* and *Zannichellia*. Proc. Calif. Acad. Sci. III. 1: 1-62. *pls.* 1-5. 1897.
2. CHAMBERLAIN, C. J., Contribution to the life history of *Salix*. BOT. GAZ. 23: 147-179. *pls.* 12-18. 1897.
3. COULTER, J. M., Contribution to the life history of *Ranunculus*. BOT. GAZ. 25: 73-88. *pls.* 4-7. 1898.
4. ——— AND CHAMBERLAIN, C. J., Morphology of angiosperms 137. 1903.
5. EICHLER, A. W., Blüthendiagramme. Erster Theil. Leipzig. 1875.
6. GUIGNARD, L., Nouvelle études sur la fécondation. Ann. Sci. Nat. Bot. VII. 14: 163-296. *pls.* 9-18. 1891.
7. ———, La double fécondation sur le *Naias major*. Jour. Botanique 15: 205-213. *figs.* 14. 1901.
8. ———, La double fécondation chez les Solanées. Jour. Botanique 16: 145-167. *figs.* 45. 1902.
9. ———, Le double fécondation chez les Crucifères. Jour. Botanique 16: 361-368. *figs.* 20. 1902.
10. HALL, J. H., An embryological study of *Limncharis emarginata*. BOT. GAZ. 33: 214-219. *pl.* 9. 1902.
11. HORN, P., Zur Entwicklungsgeschichte der Blüthe von *Elodea canadensis*. Archiv der Pharmacie III. 1. 426-433. *pl.* 1. 1872.
12. ROSENBERG, O., Ueber die Pollenbildung von *Zostera*. Meddel. Stockholms Högsk. Bot. Inst. pp. 21. 1901.
13. SCHAFFNER, J. H., A contribution to the life history and cytology of *Erythronium*. BOT. GAZ. 31: 369-387. *pls.* 4-9. 1901.
14. SHIBATA, K., Die Doppelbefruchtung bei *Monotropa uniflora* L. Flora 90: 61-66. 1902.
15. SHOEMAKER, D. N., Notes on the development of *Hamamelis virginiana* L. Johns Hopkins Univ. Cir. 21: 86-87. 1902.
16. STRASBURGER, E., Ein Beitrag zur Kenntniss von *Ceratophyllum submersum* und phylogenetische Erörterungen. Jahrb. Wiss. Bot. 37: 477-526. *pls.* 9-11. 1902.

EXPLANATION OF PLATES I-IV.

All figures were made with Bausch and Lomb camera lucida, and original drawings were reduced one-half in reproduction. Figures with magnification greater than 600 diameters were made with Zeiss apochromatic objective 2^{mm}, 1.30 N. A., and Zeiss compensating oculars 4 and 12. All others with Spencer 5^{mm} and 16^{mm} objectives and oculars 4 and 8. The original

magnifications in diameters were approximately as follows: figs. 1-22, 120; 23-42, 850; 43-66, 670; 67, 2300; 68, 69, 670; 70-74, 850; 75, 380; 76, 77, 850; 78-82, 600; 83, 30.

The abbreviations employed in describing figures are as follows: *a*, air cavity; *ar*, archesporium, *at*, antipodals; *e*, egg; *en*, endosperm nucleus; *fl*, flower; *g*, generative cell; *m*, male cell; *o*, ovule; *p*, petal; *po*, polar; *pt*, pollen tube; *s*, sepal; *sg*, synergid; *smc*, spore mother-cells; *sp*, sperm; *spt*, spathe; *st*, stem; *stg*, stigma; *stm*, stamen; *t*, tube nucleus; *v*, vesicular cell; *w*, primary wall cell.

PLATE I.

FIG. 1. Early stage in the development of pistillate flower. Flower seen at side of stem.

FIG. 2. Flower grown out, nearly equaling stem tip in length. Spathe pushing out at base of flower.

FIG. 3. Stem tip resuming original direction. Receptacle flattens.

FIGS. 4-6. Stages immediately preceding development of the floral tube. Calyx showing at margin of receptacle.

FIGS. 7-9. Early development of floral tube. Sterile stamens next to calyx.

FIG. 10. Stigmas and ovules developing simultaneously.

FIG. 11. Later stage showing beginning of corolla.

FIG. 12. Pistillate flower with principal parts all established, and floral tube entering upon period of more rapid elongation.

FIGS. 13-16. Early stages of staminate flower.

FIG. 17. Outer stamens showing next to calyx.

FIGS. 18-20. Development of stamens.

FIG. 21. Corolla growing up between calyx and outer stamens.

FIG. 22. Later stage in development of staminate flower.

PLATE II.

FIG. 23. Early stage in development of ovule, showing archesporial cell.

FIG. 24. Primary sporogenous cell and two parietal cells.

FIG. 25. Division of primary sporogenous cell.

FIGS. 26, 27. Division of daughter cells.

FIG. 28. Ovule with six megasporos. The two central cells of the row of four have each divided, making six in all. The innermost and outermost megasporos developing.

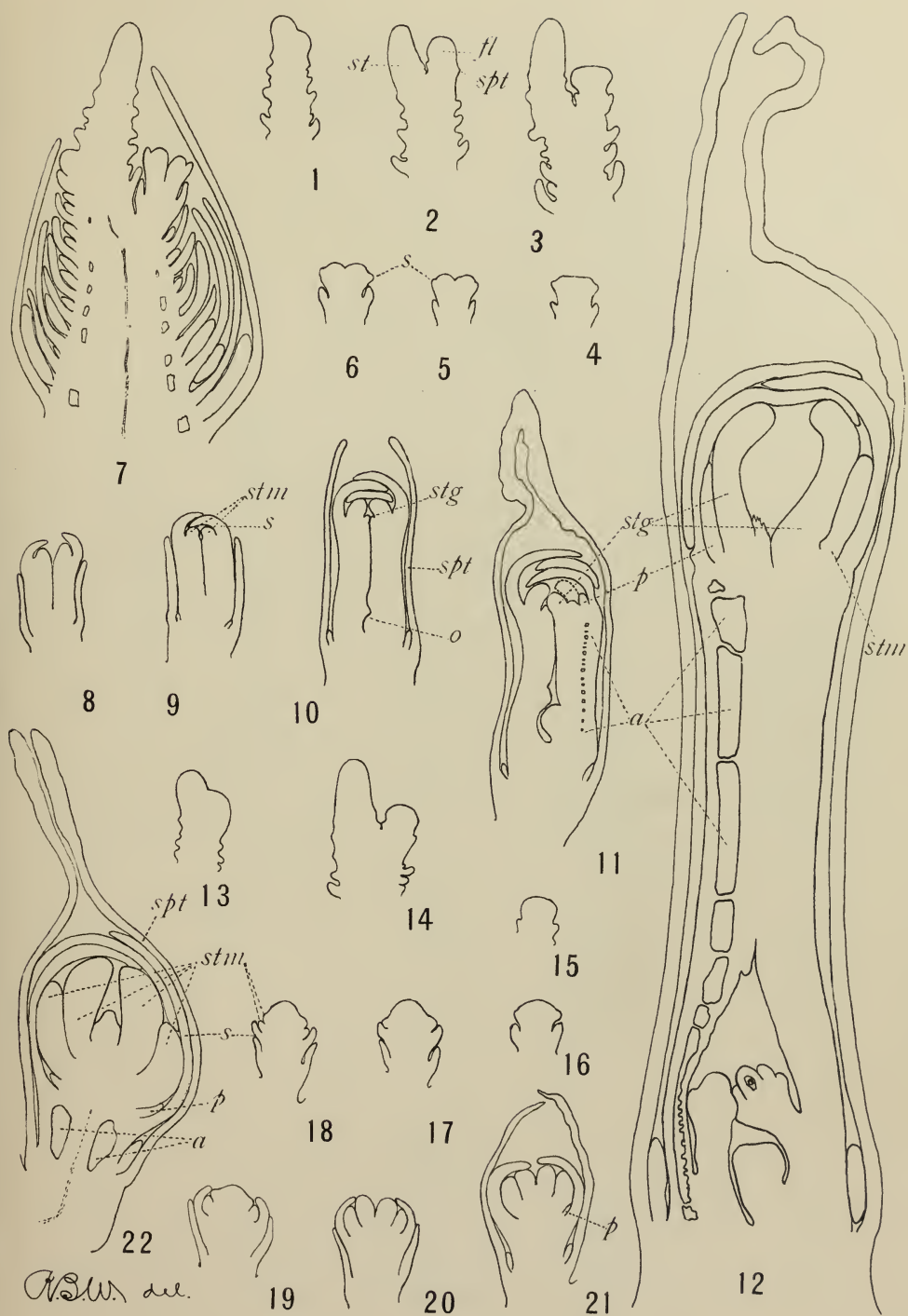
FIG. 29. Row of four megasporos. The deeper-lying one is developing and others are being crowded out.

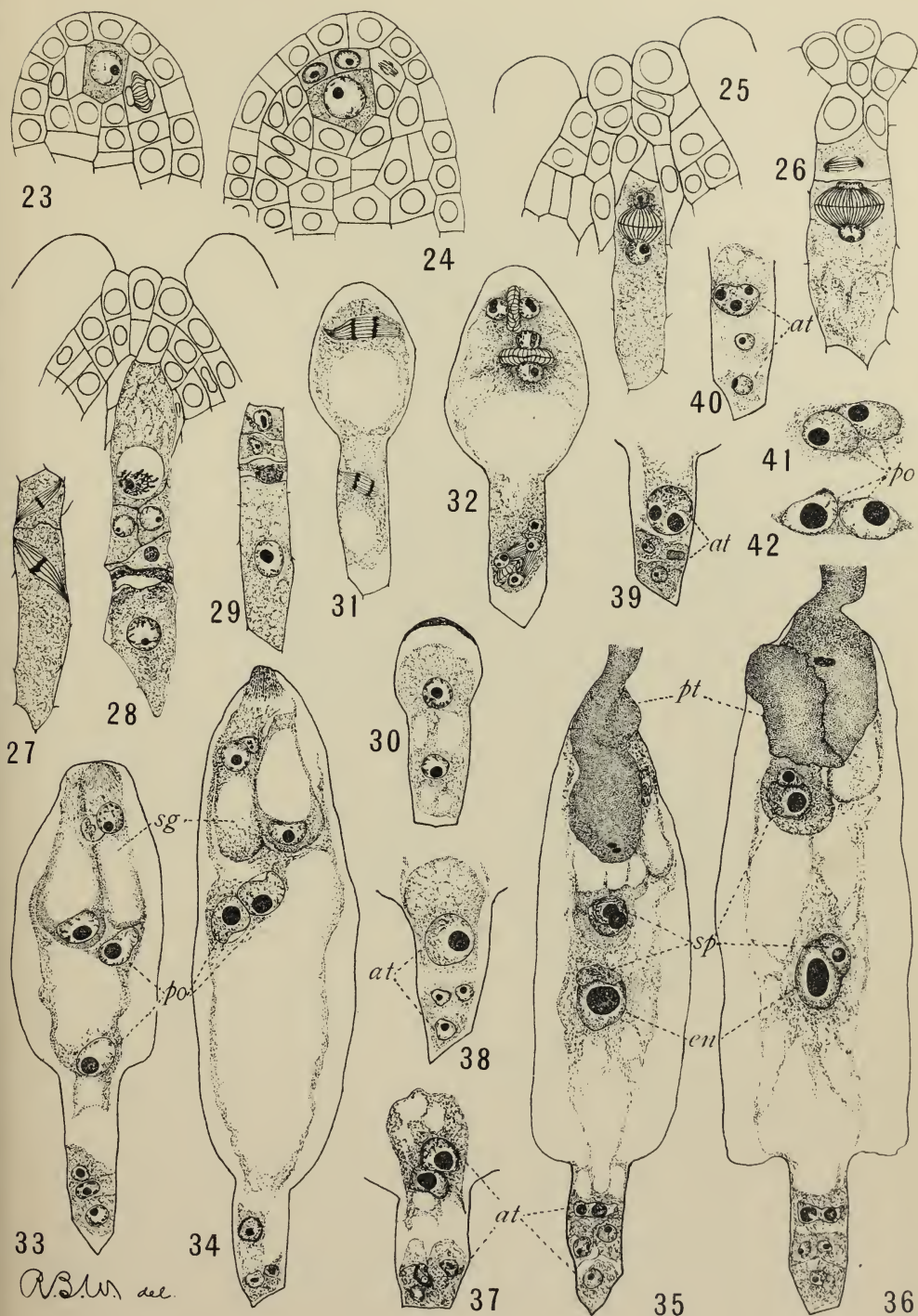
FIG. 30. Two-celled embryo sac, showing early development of antipodal pouch.

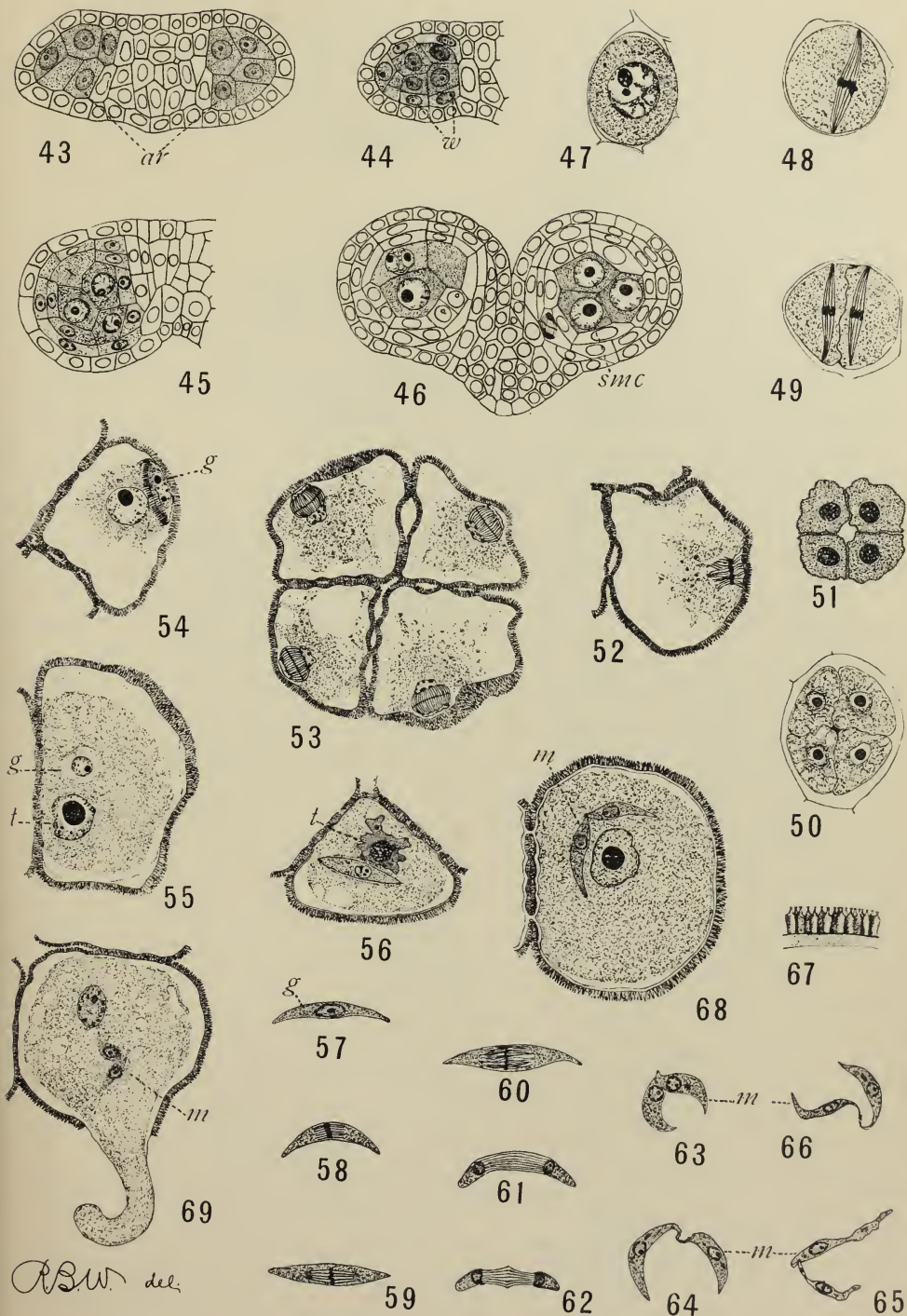
FIG. 31. Division of these nuclei.

FIG. 32. Establishment of 8-celled embryo sac.

FIG. 33. Older embryo sac showing arrangement of cells. Polars approaching one another.







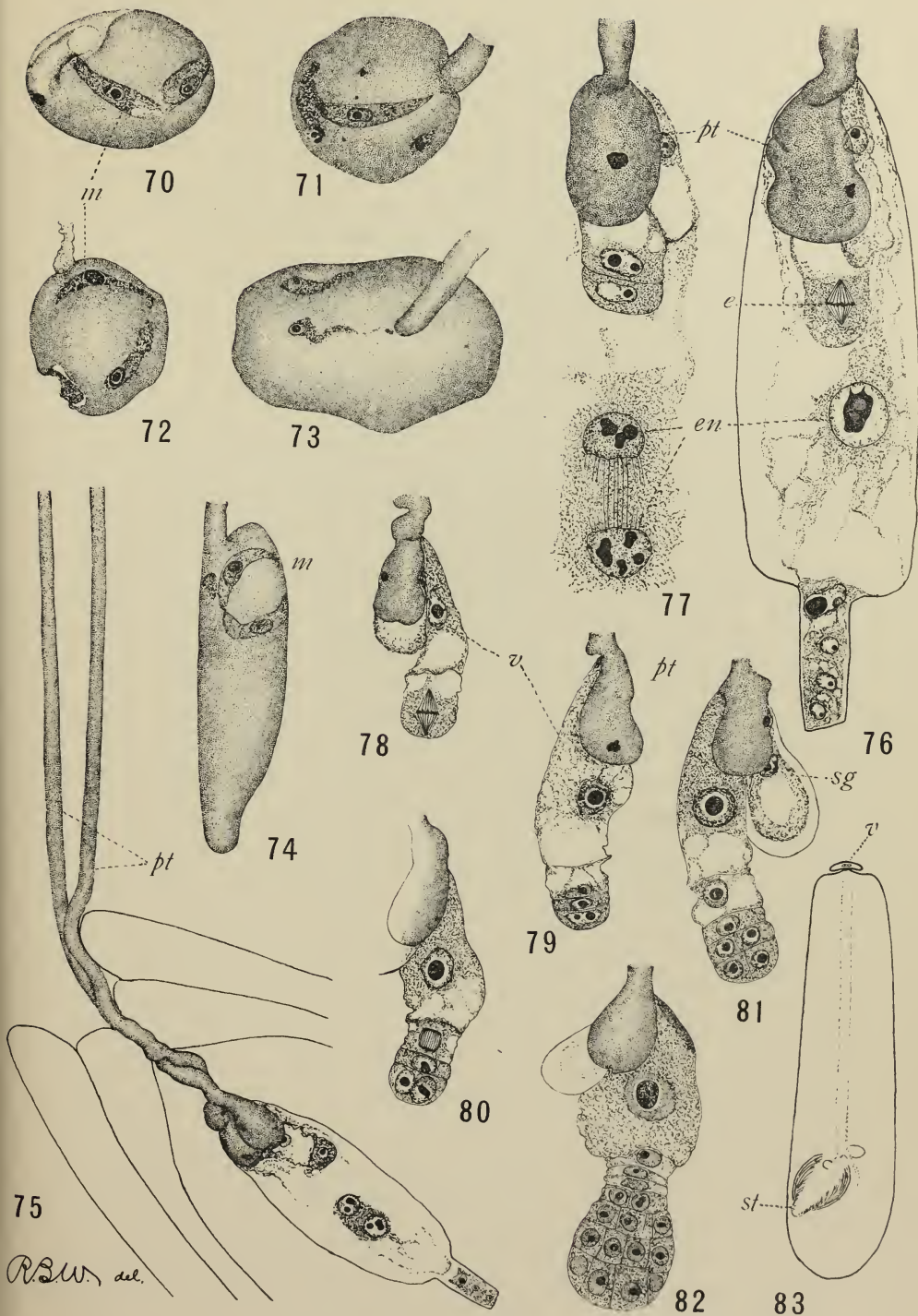


FIG. 34. Embryo sac just before fertilization. The polars lie in contact.

FIG. 35. Embryo sac at time of fertilization. One sperm lies in contact with the endosperm nucleus; the other is uniting with the egg.

FIG. 36. A similar stage. The pollen tube contents have apparently burst out of the synergid.

FIG. 37. Antipodal group showing five nuclei.

FIG. 38. Antipodal group. The enlarged nucleus surrounded by dense cytoplasm which is bounded by a membrane.

FIG. 39. An antipodal group similar to *fig. 38* without membrane about enlarged cell.

FIG. 40. An antipodal group showing three nuclei.

FIGS. 41, 42. Polars as they lie in contact before fertilization.

PLATE III.

FIG. 43. Cross-section of stamen showing the archesporia of the two sporangia.

FIG. 44. Archesporium cutting off primary wall layer which nearly invests the primary sporogenous cells.

FIG. 45. Showing several wall layers and cells cut off the sporogenous tissue on the axial side of the sporangium.

FIG. 46. Cross-section of stamen after spore mother-cells are established.

FIG. 47. Spore mother-cell immediately before division.

FIG. 48. First division of spore mother-cell.

FIG. 49. Division of daughter-cells.

FIG. 50. Young tetrad enclosed by wall of mother-cell.

FIG. 51. Young tetrad free from wall of mother-cell.

FIG. 52. Division of microspore nucleus.

FIG. 53. Four spores of tetrad clinging together with their nuclei dividing simultaneously.

FIG. 54. Generative cell being cut off along wall of spore. The tube nucleus already enlarged.

FIG. 55. Generative cell has passed into the cytoplasm of the tube cell.

FIG. 56. Tube nucleus amoeboid in form, generative cell elongated.

FIG. 57. Generative cell as it appears before division into the male cells.

FIGS. 58-62. Stages in the division of generative cell.

FIGS. 63-66. Male cells joined in pairs by their elongated ends.

FIG. 67. The spore coats; the exine with spines.

FIG. 68. A pollen grain at time of pollination, showing tube nucleus and male cells.

FIG. 69. Pollen grain germinating on the stigma of a pistillate flower.

PLATE IV.

FIGS. 70-74. Cyst-like enlargements formed at ends of pollen tubes in ovarian cavity, showing male structures are still distinct cells.

FIG. 75. Embryo sac into which two pollen tubes have entered, showing course of tube and mode of entrance into sac.

FIG. 76. Division of fertilized egg. Endosperm nucleus still in resting condition.

FIG. 77. Division of primary endosperm nucleus in embryo sac with two-celled embryo.

FIG. 78. Two-celled embryo with lower nucleus in division.

FIG. 79. Four-celled proembryo.

FIG. 80. The end cell of embryo has divided by a vertical wall.

FIG. 81. Embryo with enlarged synergid.

FIG. 82. Later stage in development of embryo showing greatly enlarged vesicular cell.

FIG. 83. Diagram of longitudinal section of embryo from nearly ripe seed. Flattened vesicular cell at root end, lateral stem tip, and secondary roots from near base of stem.



